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Air Pollution and Polyclonal Elevation of Serum Free Light Chains: An Assessment of Adaptive Immune Responses in the Prospective Heinz Nixdorf Recall Study

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Background: Residential exposure to air pollution (AP) has been shown to activate the immune system (IS). Although innate immune responses to AP have been studied extensively, investigations on the adaptive IS are scarce.

Objectives: The aim of this study was to investigate the association between short- to long-term AP exposure and polyclonal free light chains (FLC) produced by plasma cells.

Methods: We used repeated data from three examinations (t_0: 2000–2003; t_1: 2006–2008; and t_2: 2011–2015) of the population-based German Heinz Nixdorf Recall cohort of initially 4,814 participants (45–75 y old). Residential exposure to total and source-specific particulate matter (PM) with an aerodynamic diameter of 10 or 2.5 μm (PM_{10} and $PM_{2.5}$ respectively), nitrogen dioxide (NO_2), and particle number concentrations (accumulation mode; PN_{AM}) was estimated using a chemistry transport model with different time windows (1- to 365-d mean ± standard deviation) before blood draw. We applied linear mixed models with a random participant intercept to estimate associations between total, traffic- and industry-related AP exposures and log-transformed FLC, controlling for examination time, sociodemographic and lifestyle variables, estimated glomerular filtration rate and season.

Results: Analyzing 9,933 observations from 4,455 participants, we observed generally positive associations between AP exposures and FLC. We observed strongest associations with middle-term exposures, e.g., 3.0% increase in FLC (95% confidence interval: 1.8%, 4.3%) per interquartile range increase in 91-d mean of NO_2 (14.1 $\mu g/m^3$). Across the different pollutants, NO_2 showed strongest associations with FLC, followed by PM_{10} and PN_{AM}. Effect estimates for traffic-related exposures were mostly higher compared with total exposures. Although NO_2 and PN_{AM} estimates remained stable upon adjustment for PM, PM estimates decreased considerably upon adjustment for NO_2 and PN_{AM}.

Discussion: Our results suggest that middle-term AP exposures in particular might be positively associated with activation of the adaptive IS. Traffic-related PM, PN_{AM}, and NO_2 showed strongest associations. https://doi.org/10.1289/EHP7164

Introduction

Short- and long-term air pollution (AP) is associated with chronic diseases and mortality (Brook et al. 2018; Franklin et al. 2015; Newby et al. 2015). Oxidative stress and activation of the immune system are important mechanisms that are hypothesized to mediate associations of AP and chronic diseases, i.e., cardiovascular diseases. A modulation of the immune system may further lead to autoimmune diseases such as multiple sclerosis (Bosello et al. 2018), rheumatoid arthrosis (Gottenberg et al. 2007), or hypersensitivity-like responses (Redegeld et al. 2002). The immune system evolves from a complex interplay of immune cells, biological structures, and processes to defend the organism against pathogens. It can be divided into two subsystems, the innate immune system (IIS) and the adaptive (or acquired) immune system (AIS). The IIS is characterized by an unspecific immune response such as macrophage activity eliciting inflammation and activates the AIS, indicating a specific immune reaction accompanied by the synthesis of antibodies. Antibody synthesis by B-lymphocytes indicates an acquired cellular (triggered by T-lymphocytes) or humoral immune reaction against specific antigens, e.g., particulate matter (PM).

It has been hypothesized that the IIS and AIS interact substantially in reaction to PM exposure (Miyata and van Eeden 2011). The recognition of air pollutants by toll-like receptors may lead to the activation of signaling pathways involving transcription factors like nuclear factor κ B (NF-κ B, which then promotes antigen presentation and activation of plasma cells (Miyata and van Eeden 2011). Although associations between AP and markers of innate (unspecific) immune responses like C-reactive protein (CRP) have been studied extensively (Rückerl et al. 2011; Hennig et al. 2014), epidemiological studies on stimulation of the AIS by AP are scarce. A previously published study in China supports a link by suggesting that $PM_{2.5}$ (PM with an aerodynamic diameter ≤2.5 μm) exposure may also directly stimulate elements of the AIS including both T and B cells. Investigating the immunological consequences of air pollutant exposure in a cohort of 110 Chinese traffic policemen, Zhao et al. (2013) observed that high levels of 24-h $PM_{2.5}$ were linked with increased IgM, IgG, and IgE plasma concentrations, indicating a stimulation of the humoral immune system. These findings are consistent with results from an earlier cross-sectional study on schoolchildren that found associations of long-term exposure to $PM_{2.5}$ with higher levels of biomarkers of cellular and humoral AIS response (Leonardi et al. 2000). Prior studies indicate a particularly adverse role of traffic-related AP in the context of immune responses (Zhao et al. 2013; Kerkhof et al. 2010).

Serum free light chains (FLC) can be measured in blood serum and serve as a marker of the activation of the AIS (Brebner

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and Stockley 2013). Structurally, an antibody consists of two heavy and two light chains. During antibody synthesis in plasma cells, excess light chains are secreted into the blood as FLC. Each plasma cell produces only one isotype of FLC: kappa (κ) or lambda (λ) with a normal production ratio of 2:1. Polyclonal elevation of FLC, characterized by an increased production of both κ and λ without an abnormal κ:λ ratio, may identify unspecific immune activation of the AIS. In healthy adults, serum FLC are rapidly cleared by the kidneys, i.e., within 6 h. However, chronic AP exposure may modulate the immune system in various ways, eliciting airway inflammation accompanied by various responses of the immune system (Grove et al. 2018).

In our present study, we investigated exposure to total and source-related $PM_{2.5}$, PM_{10}, accumulation-mode particle number concentration (PN_{AM}, between 0.1 and 1.0 μm in aerodynamic diameter), and NO_2 in association with polyclonal serum FLC, measured as the sum of κ and λ FLCs, in the general population free of monoclonal disorders. AP was estimated with a spatiotemporal chemical-transport model for various time windows.

We further investigated high-sensitivity CRP (hs-CRP), prevalent diabetes mellitus (DM), prevalent coronary heart disease (CHD), estimated glomerular filtration rate (eGFR), and other characteristics as potential effect modifiers. We used data from baseline and two follow-up observations on AP exposure and FLC, repeatedly measured in the Heinz Nixdorf Recall Study, a population-based prospective cohort from the Ruhr area in North Rhine-Westphalia, Germany.

Methods

Study Design

We used data from the baseline (t_0: 2000–2003), 5-y follow-up (t_1: 2006–2008), and 10-y follow-up (t_2: 2011–2015) examinations of the Heinz Nixdorf Recall (HNR) study. Study participants were randomly selected from registries of the local residents' registration offices of three large adjacent cities (Mülheim, Essen, and Bochum) in the German Ruhr area (recruitment efficacy proportion: 55.8; Stang et al. 2005). A total of 4,814 participants completed the baseline study visit in 2000–2003 (mean age: 59.4 y), of which 4,157 and 3,089 also completed the 5- and 10-y follow-up visits, respectively. Assessment for baseline and follow-up examinations included a self-administered questionnaire, face-to-face interviews for personal risk factor assessment, clinical examinations, and comprehensive laboratory tests according to standard protocols. Details on the study design have been described elsewhere (Schmermund et al. 2002). The HNR study was approved by the institutional ethics committee of the University Duisburg-Essen and the University Hospital of Essen and followed strict internal and external quality assurance protocols. All participants gave informed consent.

Exposure Assessment

Hourly mass concentrations for PM_{10},$PM_{2.5}$, and NO_2 and hourly number concentrations of PN_{AM} were estimated by means of the validated European Air Pollution Dispersion chemistry transport model (EURAD) (Memmesheimer et al. 2004). The multigrid EURAD model predicts AP exposures by considering transport, chemical transformation, and the deposition of tropospheric constituents. Based on sequential nesting from mid-Europe to central Europe over North Rhine-Westphalia (NRW) to the southwestern part of the Ruhr area (horizontal grids of 125 km, 25 km, 5 km, and 1 km), it includes data from official emission inventories, hourly meteorology, and regional topography. Emission inventories contribute data on different source categories as transport and industry, according to the Selected Nomenclature for Sources of Air Pollution (SNAP). Output measures of the EURAD-model include particle mass, particle number concentrations, and other pollutants per day and grid.

An assimilation procedure for PM_{10} and NO_2 estimates was performed by integrating the modeled exposure values with measured air pollutant data routinely collected at the monitoring sites in the study area by the local environmental agency (State Agency for Nature, Environment, and Consumer Protection). Due to a lack of routinely measured data, the assimilation process could not be applied to $PM_{2.5}$ and PN_{AM} (Nonnemacher et al. 2014).

In addition to total exposures, the EURAD model estimates industry- and traffic-specific exposures to PM and PN_{AM}, based on specific emission factors. Single emission categories can be set to zero to study the impact of single sources on AP concentrations. This method was applied to examine local traffic- and local industry-related exposure concentrations within the study area. To this end, the model was first run including the complete emission input (e.g., PM_{total}). In a second step, the model was run with the complete emission input excluding local traffic ($PM_{no\text{-}traffic}$). Finally, $PM_{no\text{-}traffic}$ was subtracted from PM_{total} to define traffic-related exposure concentrations. The same method was used to calculate industry-related exposure concentrations (Hennig et al. 2016).

Within a study area covering $\sim 600\,km^2$, mean daily air pollutant concentrations were assigned to the participants' addresses on a horizontal grid resolution of $\sim 1\,km^2$ using ArcView 9.2 (Nonnemacher et al. 2014), considering various short- to long-term mean exposure windows of 1 to 365 d before the examination date of baseline, 5-y, and 10-y follow-up study. Short-term meteorological data including humidity, temperature, and wind speed in the North/South and East/West directions were also assigned by grid.

Assessment of FLC

FLC measurements for baseline, 5-y, and 10-y follow-up evaluation were available from a previous study (Eisele et al. 2013) and performed on a Dade Behring BN-II automated nephelometer (Siemens) using a commercially available kit (FreeLite, The Binding Site Ltd.). Blood samples were processed directly after the blood draw and stored at −80°C (Eisele et al. 2013). FLC concentrations in serum samples were measured on the same device and in the same laboratory. An abnormal κ:λ FLC ratio was defined as <0.26 or >1.65 (Katzmann et al. 2002).The sum of κ and λ in milligrams per liter was then used as a measure for polyclonal serum FLC.

Definition of Covariates

Individual socioeconomic status (SES) was determined by the total years of education, based on the International Standard Classification of Education (UNESCO 1997) and divided into four categories (≤10y, 11–13 y, 14–17 y, ≥18 y). Administrative data on unemployment rates of the 106 administrative neighborhoods (median size of 11,263 inhabitants) were used (Dragano et al. 2009) to define neighborhood based SES (nSES). Smoking variables included smoking status (current, former, and never smoker based on the past year), cumulative packyears at baseline examination and environmental tobacco smoke (ETS) at work and/or at home. Physical activity (yes/no) referred to regular weekly exercise for at least 30 min. Alcohol consumption was derived from self-declared details on consumed drinks per week and was classified in quartiles of pure alcohol consumption in grams per examination and sex. Anthropometric (size, weight,

and waist circumference) and blood pressure measurements were conducted according to standardized protocols. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in metres) squared. DM was defined as prior physician diagnosis of DM or taking an antidiabetic drug. Standard enzymatic methods were used to measure hs-CRP in EDTA plasma (Schmermund et al. 2002). Estimated glomerular filtration rate (eGFR) was calculated with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Creatinine Equation (Levey and Stevens 2010). All anthropometric and biomarker characteristics were examined equally at baseline and/or follow-up examinations, except alcohol consumption, where the examination technique changed from self-declared questionnaire at baseline to computer-based interview at follow-up examinations. At baseline, CHD was defined as a self-reported history of a myocardial infarction or coronary intervention. Incident CHD during follow-up was based on self-reported incident coronary events that met predefined study criteria (Schmermund et al. 2002), confirmed with medical records by an independent end point committee (Erbel et al. 2010). Season (spring, summer, fall, and winter) was modeled according to meteorological seasons.

Analytical Strategy

The association between AP and log-transformed FLC for different exposure windows preceding examinations was investigated by applying mixed linear models including a random participant intercept to account for the correlation of repeated measures of baseline (t_0), first (t_1) and second (t_2) follow-up examinations. Cumulative exposure-time windows ranging from 1 to 365 d were chosen because of biological evidence for short (FLC) to middle (B-lymphocytes) renewal cycles. In addition, seasonally dependent AP exposures were likely to play a role. As an exploratory analysis, longer-term exposure-windows were also examined.

Based on biological and epidemiological evidence, we built different adjustment sets to account for potential confounding. In our main model, we included the exposure, examination time and season, age, sex, BMI, SES, nSES, lifestyle variables (smoking status and pack years, ETS, physical activity, and alcohol intake), and eGFR. We tested continuous covariates of the model for linearity and as a result included age as quadratic term. eGFR was included as z-scored, centered values for the three examination times in the model. The linearity of the exposure–response relationship was explored by introducing natural cubic splines with different degrees of freedom and comparing the respective Akaike information criterion (AIC; a measure of goodness of fit) of the models. Two-pollutant models were built including copollutants of the same time window. Effect estimates are presented as percent change in serum FLC per interquartile range (IQR) (75th–25th quartile) of AP. For source-specific exposure models, we present effect estimates per 1 $\mu g/m^3$ PM and NO_2 as well as per 500 PN_{AM}. Analyses were performed in R [version 3.6.2 (http://cran.r-project.org/)].

Effect Modification

We evaluated effect modification by including interaction terms between each PM exposure (modeled as a continuous variable), dichotomous variables for sex (females, males), age (<65 y, ≥65 y), BMI (<30 kg/m^2, ≥30 kg/m^2), DM (Ma et al. 2012), CHD, eGFR (dichotomized at 60 ml/min), hs-CRP (<0.3, ≥0.3), and categorical variables for smoking (never smoker, former smoker, and smoker), educational level (low/high), and season. Likelihood ratio tests ($\alpha = 0.05$) were used to assess stratum-specific statistical significance.

Further Sensitivity Analysis

To assess different covariance patterns in our model, we applied the general least squared function (GLS) to test different covariance patterns in our model. According to the AIC, the unstructured model showed the best model fit, resulting in similar point estimates as our mixed linear models. In addition to consider cumulative exposure concentrations, we considered delayed effects of exposure concentrations for the 12 months prior to the examination, ranging from day 1-31 up to day 341-372. Moreover, we adjusted main models for short-term temperature (28-d mean) instead of season. In addition, we tested the model further by including more meteorological variables from 1 up to 91-d mean averaging periods. To explore optimal meteorological time windows (1- to 14-d), the different variables were included in an exposure-free model. Following this procedure, 1-d mean meteorological variables were selected. Seasonality was further investigated by introducing day of year as a penalized spline with six degrees of freedom. To evaluate the robustness of our main analysis regarding examination time, we estimated exposure effects on FLC at the three examination times separately (t_0, t_1, and t_2). To gain more insight into potential associations with specific isotypes of FLCs, we ran our main model for FLC isotypes κ and λ separately. To analyze the influence of the included variables, we compared different adjustment sets. Extended models were additionally adjusted for city. Finally, to account for the considerable loss to follow-up between baseline and second follow-up examinations, we further conducted a sensitivity analysis to compare participants taking part in the baseline and in the first follow-up examination but not in the second follow-up examination with all other participants by applying inverse probability weights. Models employing stabilized weights were used to predict loss to follow-up with age, sex, BMI, individual SES, smoking status and history, alcohol consumption, physical activity, renal function, and season as predictors.

Results

Study Population

There were 11,241 observations from 4,617 study participants without known monoclonal gammopathy of undetermined significance (Eisele et al. 2012), documented lymphoma or an abnormal FLC ratio (Figure S1). We excluded observations from participants with missing information on outcome, exposure, or who had moved outside the study area (980 observations). Finally, we excluded observations from participants with missing data on covariates (328 observations), resulting in a study sample of 9,933 observations from 4,455 participants.

Characteristics of the Study Population

Population characteristics are described for the three examination times separately, including 4,174 participants at t_0 (49.5% females; mean ± standard deviation (SD) age 59.4 ± 7.8), 3,481 participants at t_1 (51.1% females; age 64.3 ± 7.6), and 2,278 participants at t_2 (51.8% females; age 68.7 ± 7.3; Table 1). Over time, we observed fewer current smokers as well as, higher prevalence of DM, statin intake, and CHD, whereas BMI and hs-CRP did not change considerably. For passive smoking and κ-FLC, we observed a dip at t_1. For eGFR and alcohol consumption, we observed a shift between baseline and follow-up examinations toward lower (eGFR) and higher (alcohol) values. Baseline observations excluded due to missing data in the main analysis ($n = 640$) compared with included participants ($n = 4,174$) showed similar AP exposures, FLC concentrations, and education levels, but lower current and passive smoking rates and alcohol

Table 1. Characteristics of the Heinz Nixdorf Recall Study population at baseline (2002–2003), first (2006–2008), and second (2011–2015) follow-up examinations ($n = 4{,}455$ participants).

Characteristic	Baseline ($n = 4{,}174$)	1st Follow-up ($n = 3{,}481$)	2nd Follow-up ($n = 2{,}278$)
Age (mean±SD)	59.5 (7.8)	64.3 (7.6)	68.7 (7.3)
Sex, female [*n* (%)]	2,067 (49.5)	1,778 (51.1)	1,180 (51.8)
Employment status [*n* (%)][a]			
Employed	1,712 (41.0)	1,135 (32.6)	486 (21.5)
Inactive or housewife	562 (13.5)	301 (8.6)	111 (4.9)
Pensioner	1,633 (39.2)	1,911 (54.9)	1,595 (70.5)
Unemployed	264 (6.3)	133 (3.8)	71 (3.1)
Education level [*n* (%)]			
≤10 y	462 (11.1)	355 (10.2)	206 (9.0)
11–13 y	2,325 (55.7)	1,966 (56.5)	1,283 (56.3)
14–17 y	937 (22.4)	765 (22.0)	522 (22.9)
≥18 y	450 (10.8)	395 (11.3)	267 (11.7)
Neighborhood unemployment (mean±SD)	12.5 (3.4)	12.4 (3.4)	12.3 (3.4)
BMI (mean±SD)	27.9 (4.6)	28.3 (4.9)	28.2 (4.7)
Smoking status [*n* (%)]			
Never smoker	1,730 (41.4)	1,471 (42.3)	1,009 (44.3)
Ex-smoker	1,444 (34.6)	1,397 (40.1)	980 (43.0)
Current smoker	1,000 (24.0)	613 (17.6)	289 (12.7)
Packyears [mean±SD]	27,7 (27.1)		
ETS exposure, yes [*n* (%)]	1,549 (37.2)	863 (24.8)	1,341 (58.9)
Alcohol consumption per wk in g; median (IQR)	12.9 (61.8)	22.2 (78.4)	21.8 (119.1)
FLC (mg/L); median (IQR)			
Kappa (κ)	15.0 (5.9)	12.0 (5.2)	14.3 (6.3)
Lambda (λ)	15.1 (6.0)	14.5 (5.7)	14.1 (5.3)
Total (κ+λ)	30.1 (11.2)	26.6 (10.1)	28.4 (10.9)
hs-CRP [median (IQR)][b]	0.2 (0.2)	0.1 (0.2)	0.1 (0.2)
Statin intake, yes [*n* (%)][c]	411 (10.5)	691 (19.9)	630 (27.7)
eGFR (mean±SD)	79.3 (14.6)	64.8 (11.5)	63.3 (12.2)
CHD, yes [*n* (%)][d]	271 (6.5)	289 (8.3)	224 (9.8)
Diabetes, yes [*n* (%)]	342 (8.2)	481 (13.8)	357 (15.7)
Season [*n* (%)]			
Spring	1,144 (27.4)	958 (27.5)	444 (19.5)
Summer	1,122 (26.9)	776 (22.3)	696 (30.6)
Autumn	931 (22.3)	872 (25.1)	670 (29.4)
Winter	977 (23.4)	875 (25.1)	468 (20.5)

Note: BMI, body mass index; CHD, coronary heart disease; eGFR, estimated glomerular filtration rate; ETS, environmental tobacco smoke; FLC, free light chains; hs-CRP, high sensitivity C-reactive protein; IQR, interquartile range; SD, standard deviation.
[a]Missing observations for Baseline: 3, 1st Follow-up: 1, 2nd Follow-up: 1.
[b]Missing observations for Baseline: 11, 1st Follow-up: 74, 2nd Follow-up: 3.
[c]Missing observations for Baseline: 270.
[d]Missing observations for Baseline: 7.

consumption (Table S1). Although mean residential $PM_{2.5}$ and NO_2 exposure concentrations decreased steadily from baseline (2002–2004) to the second follow-up examination (2012–2014), exposure concentrations of total PM_{10} and PN_{AM} decreased between baseline and first follow-up examination but increased between first and second follow-up examinations to concentrations similar to or slightly below baseline (Table 2; Table S2). In general, traffic- and industry-specific exposures decreased over time. Correlations of 91-d mean exposures ranged from 0.19 to 1.00 with highest correlations within sources (Table S3). NO_2 was generally highly correlated with total PM and all traffic-related exposures (0.58–0.80). Correlation between hs-CRP and FLC was low, with Spearman correlation coefficient of 0.2.

Association of PM with FLC

The evaluation of the exposure-response relationship indicated linear (PM) or a slightly quadratic (PN_{AM} and NO_2) relationships in the fully adjusted 91-d mean exposure models (Figure S2). In the main model, an IQR increase in total pollutant was associated with increased FLC concentrations across all pollutants and nearly all middle- to long-term exposure windows (Figure 1; Table 3; Table S4). Effect estimates were highest around 91-d mean concentrations; showing strongest associations for NO_2 [3.0% (95% confidence interval (CI): 1.8%, 4.3%] per 14.1 $\mu g/m^3$ increase in 91d-mean exposures). Analyzing different adjustment sets, effect estimates were strongly affected by season and examination time, being most obvious for PM (Figure S3; Table S5) and decreased further upon adjustment for demographic and lifestyle variables. Most effect estimates (except NO_2 estimates) were also sensitive to adjustment for eGFR. Adjustment for city of residence decreased effect estimates for PM further, whereas associations with NO_2 and PN_{AM} exposures were less affected. Comparing different exposure sources, traffic-related middle-term PM exposures yielded considerably higher effect estimates than total or industry-specific exposures (Table 3). For example, an IQR increase in 91-d mean traffic-specific PM_{10} of 0.4 $\mu g/m^3$ was associated with a 1.9% (95% CI: 1.0%, 2.9%) increase of FLC vs. 0.4% (95% CI: −0.3%, 1.1%) per 1.8 $\mu g/m^3$ increase of industry-specific and 1.3% (95% CI: 0.4%, 2.2%) per 5.9 $\mu g/m^3$ increase of total PM_{10}. For short-term (3-d mean) exposures, effect estimates decreased (total and traffic-related PM, PN_{AM} and NO_2) or increased slightly (industry-related PM). For long-term (365-d mean) time windows exposures, effect estimates decreased (total and traffic-related exposures) or became negative (industry-related exposures). Models comparing effects per fixed increment of 1 $\mu g/m^3$ (PM) or per number concentrations of 500 (PN_{AM}) showed similar and more distinct results (Table S6). Although effect estimates of middle-term PM were considerably increased in relation to traffic-specific exposures compared with industry-specific and total exposures, e.g., 5.4% (2.6%; 8.3%) vs. 0.2% (−0.2%; 0.6%) and 0.2% (0.1%; 0.4%) for PM_{10}, middle-term PN_{AM} estimates were elevated for both traffic-

Table 2. Distributions of residential concentrations of 91-d mean exposure to total $PM_{2.5}$, PM_{10}, PN_{AM}, and NO_2 and source-specific exposure to $PM_{2.5}$, PM_{10}, and PN_{AM} for baseline (2001–2002), first (2006–2008), and second (2011–2015) follow-up examinations from the EURAD model.

	Baseline ($n = 4,157$)					1st Follow-up ($n = 3,481$)					2nd Follow-up ($n = 2,278$)				
Exposure, source	Min	P25	Mean±SD	P75	Max	Min	P25	Mean±SD	P75	Max	Min	P25	Mean±SD	P75	Max
$PM_{2.5}$ ($\mu g/m^3$)															
Total	12.3	15.4	17.5 (2.7)	19.3	27.3	9.6	13.5	15.9 (3.0)	18.0	25.2	7.7	11.0	14.5 (4.0)	17.2	26.8
Traffic	0.1	0.6	0.8 (0.3)	1.0	2.4	0.1	0.4	0.6 (0.2)	0.7	1.4	0.1	0.3	0.4 (0.1)	0.5	1.1
Industry	0.1	0.8	1.6 (1.0)	2.1	9.2	0.3	0.8	1.5 (0.9)	2.0	6.1	0.3	9,7	1.4 (0.9)	1.8	11.1
PM_{10} ($\mu g/m^3$)															
Total	13.2	18.3	21.1 (3.9)	23.4	36.3	10.6	16.8	19.5 (4.3)	22.4	34.9	11.2	16.9	21.0 (5.1)	24.7	38.0
Traffic	0.1	0.6	0.8 (0.3)	1.0	2.6	0.1	0.4	0.6 (0.2)	0.7	1.4	0.1	0.3	0.4 (0.1)	0.5	1.0
Industry	0.1	1.2	2.4 (1.6)	3.1	14.2	0.4	1.0	2.1 (1.3)	2.8	9.2	0.3	1.0	1.9 (1.3)	2.6	11.1
PN_{AM} (n/mL)															
Total	1,395.3	2,731.7	3,691.1 (349.0)	4,309.0	9,775.4	1,457.1	2,348.1	3,017.1 (838.7)	3,552.9	6,781.3	1,155.2	2,685.1	3,172.5 (793.6)	3,634.9	7,520.5
Traffic	53.8	213.8	422.6 (284.6)	573.8	1,962.1	46.8	191.2	295.1 (172.5)	341.7	1,659.8	44.7	184.3	280.0 (140.0)	338.0	1,004.0
Industry	6.8	184.2	277.4 (122.2)	353.1	937.0	68.2	190.4	274.5 (108.0)	342.4	735.0	45.8	177.8	256.3 (108.3)	318.7	909.0
NO_2 ($\mu g/m^3$)															
Total	20.7	32.4	40.9 (10.6)	48.5	76.7	18.1	30.7	38.0 (9.0)	44.8	65.9	15.5–52.4	27.2	31.7 (6.0)	36.6	52.4

Note: EURAD, European Air Pollution Dispersion; Max, maximum; Min, minimum; NO_2, nitrogen dioxide; $PM_{2.5}$, particulate matter with aerodynamic diameter ≤2.5 μm; PM_{10}, particulate matter with aerodynamic diameter ≤10 μm, PN_{AM}: accumulation mode particle number; SD, standard deviation.

and industry-specific exposures [3.9% (95% CI: 2.5%, 5.4%) and 4.1% (95% CI: 1.7%, 6.7%) vs. total exposures 0.7% (95% CI: 0.4%, 1.0%)]. Two-pollutant-models for 91-d mean NO_2 and PN_{AM} exposures mostly resulted in similar or slightly reduced effect estimates upon adjustments for PM, whereas PM estimates were reduced to null or negative estimates upon adjustment for NO_2 or PN_{AM} (Figure 2; Table S7). In contrast, associations with PN_{AM} and NO_2 were only slightly reduced upon mutual adjustments.

Effect Modification

Generally, analyses indicated an evidence for effect modification across all 91-d mean air pollutants by eGFR, hs-CRP, prevalent CHD, statin intake and season as well as by physical activity in case of $PM_{2.5}$ and DM in case of NO_2 ($p < 0.05$; Figure 3; Tables S8–S11). Estimates tended to be greater among participants with hs-CRP-values >0.3, with eGFR ≤60, prevalent CHD, and statin intake. Participants examined during autumn and winter generally showed stronger associations compared with those examined during spring and summer. For other time windows, we observed mostly similar effect modifications.

Sensitivity Analysis

Assessing delayed effects of exposures from 1 (1–31 d) to 12 months (342–372 d) prior to the examination in the main models (Figure S4; Table S12) showed associations for all 32- to 62-d lagged exposures as well as for 62- to 93-d lagged PN_{AM} and NO_2 exposures. Lagged PM exposures additionally showed associations for 197- to 217-d exposures. Lagged exposures beyond 280 d prior to examination resulted in negative effect estimates, being significant for all 342- to 372-d lagged exposures. When adjusting for 28-d mean temperatures instead of season, the general pattern remained for all pollutants and effect estimates changed to a minor extent (Figure S5; Table S13). Furthermore, 1- to 120-d mean PM exposures yielded stronger associations, whereas 182- to 270-d mean PM exposures yielded weaker associations. Associations with PN_{AM} exposures were generally weaker for 28- to 182-d mean exposures, whereas NO_2 showed mostly stronger associations. Introducing short-term averaged meteorological variables into the model resulted in slightly stronger associations for short-term (1- and 3-d means) and in weaker associations for middle-term (28- to 91-d mean) PM and PN_{AM} exposures, whereas associations with NO_2 were slightly stronger for all time windows (Figure S6; Table S14). When including a penalized spline for day of year, associations for 91-d mean PM and NO_2 exposures were stronger in comparison with the main model (Table S15). Short-, long-term PM exposures as well as PN_{AM} exposures remained similar. When stratifying our analysis set into the three examination times, we observed more distinct associations for 91-d mean exposures at baseline and second follow-up examinations. These associations were most obvious for PM_{10} and PN_{AM} (Figure S7; Table S16). When comparing specific types of FLC, effects were slightly higher for λ in association with middle-term $PM_{2.5}$ and PN_{AM} (Figure S8, Table S17). For example, we observed a 2.0% increase (95% CI: 1.3%, 2.7%) for λ-FLC compared with a 1.3% increase (95% CI: 0.5%, 2.0%) per 91-d mean PN_{AM} IQR exposure for κ-FLC. In general, all long-term exposures showed stronger associations for λ-FLC in comparison with κ-FLC; e.g., an IQR increase in 365-d mean PM_{10} resulted in elevated λ-FLC of 0.8% (95% CI: −0.0%, 1.7%) vs. 0.3% (95% CI: −0.6%, 1.2%). The application of inverse probability weights to account for attrition yielded similar, slightly increased effect estimates (Table S18).

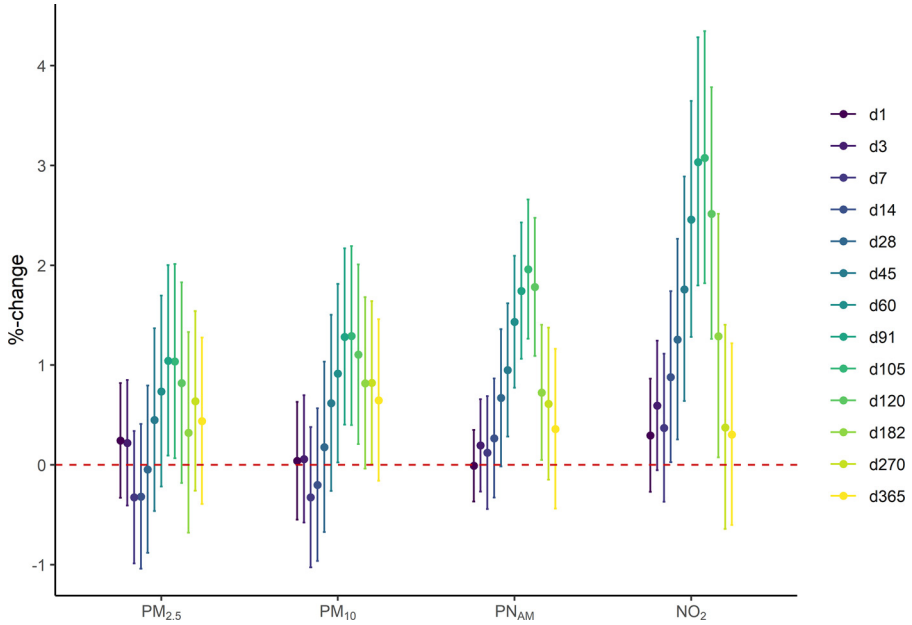

Figure 1. Associations between an interquartile range increase in mean air pollution exposure and serum free light chains as percent change, using different short- to long-term time air pollution time windows in the main models, adjusted for age, sex, education, neighborhood unemployment rate, season, examination time, smoking status, pack years, environmental tobacco smoke, BMI, physical activity, alcohol intake and renal function in 9,933 observations from 4,455 participants. Estimates are stratified by air pollutant (NO_2, $PM_{2.5}$, PM_{10}, and PN_{AM}). Time windows relate to cumulative average exposure concentrations from 1 calendar day up to the 365 calendar days preceding the examination. See Table S4 for corresponding numeric data. Note: BMI, body mass index; NO_2, nitrogen dioxide; $PM_{2.5}$, particulate matter with aerodynamic diameter less than or equal to 2.5 micrometers; PM_{10}, particulate matter with aerodynamic diameter less than or equal to 10 micrometers; PN_{AM}, accumulation mode particle number.

Discussion

Exploring various short- to long-term time windows for the association between residential exposure to $PM_{2.5}$, PM_{10}, and PN_{AM} as well as total NO_2 and polyclonal serum FLC, this study suggests that medium-term AP exposure is positively associated with an increase in polyclonal serum FLC, thus indicating activation of cells of the AIS. Across the different sources, industry-related exposures showed generally stronger effects for short time windows (3-d mean), whereas traffic-related exposures resulted in increased FLC concentrations for middle-term time windows (91-d mean). Associations for NO_2 and PN_{AM} were robust upon adjustment for PM, whereas PM estimates decreased considerably upon adjustment for NO_2 and PN_{AM}. Effect modification was obvious for, among others, prevalent CHD and hs-CRP as a marker for increased activation of the innate immune system.

Biological Mechanisms Linking AP to the Adaptive Immune System

Recent toxicological literature stresses the role of PM in eliciting a proinflammatory cascade including both the AIS and the IIS (Miyata and van Eeden 2011). The specific mechanisms of the responses to AP are still unknown. First, responding alveolar macrophages and dendritic cells are hypothesized to moderate the immune-modulating process (Hiraiwa and van Eeden 2013; Provoost et al. 2010; Grunig et al. 2014). Toxicological studies suggest that the IIS-related transcript factor NF-κB initiates

Table 3. Associations between an interquartile range increase in mean air pollution exposure in total and source-related $PM_{2.5}$, PM_{10}, and PN_{AM} and FLC in percent changes, using one short- (3-d), one middle- (91-d) and one long-term (365-d) time window in the main models, adjusted for age, sex, education, neighborhood unemployment rate, season, examination time, smoking status, environmental tobacco smoke, BMI, physical activity, alcohol intake, and renal function ($n = 9{,}933$ observations from 4,455 participants).

	3-d mean±SD exposures			91-d mean exposure			365-d mean±SD exposures		
Exposure, Source	IQR	Estimate (95% CI)	*p*-Value	IQR	Estimate (95% CI)	*p*-Value	IQR	Estimate (95% CI)	*p*-Value
$PM_{2.5}$ [μg/m³]									
Total	9.7	0.22 (−0.41, 0.85)	0.49	4.3	1.04 (0.09, 2.00)	0.03	2.3	0.44 (−0.39, 1.28)	0.30
Traffic	0.5	0.20 (−0.23, 0.64)	0.36	0.4	1.93 (0.95, 2.91)	0.00	0.4	0.19 (−0.94, 1.33)	0.75
Industry	1.6	0.49 (0.01, 0.97)	0.05	1.2	0.42 (−0.30, 1.16)	0.25	1.3	−0.42 (−1.24, 0.40)	0.31
PM_{10} [μg/m³]									
Total	11.2	0.06 (−0.58, 0.70)	0.86	5.9	1.28 (0.40, 2.17)	0.00	4.0	0.65 (−0.16, 1.46)	0.12
Traffic	0.5	0.17 (−0.27, 0.61)	0.45	0.4	1.94 (0.95, 2.94)	0.00	0.4	0.19 (−0.95, 1.35)	0.75
Industry	2.4	0.52 (0.03, 1.01)	0.04	1.8	0.38 (−0.34, 1.11)	0.30	2.0	−0.42 (−1.23, 0.41)	0.32
PN_{AM} [n/mL]									
Total	3,051.2	0.19 (−0.27, 0.66)	0.41	1,236.3	1.74 (1.06, 2.43)	0.00	710.2	0.36 (−0.44, 1.16)	0.37
Traffic	243.2	0.08 (−0.08, 0.25)	0.33	208.2	1.61 (1.03, 2.20)	0.00	140.6	0.54 (−0.25, 1.33)	0.18
Industry	297.5	0.53 (0.05, 1.01)	0.03	156.1	1.28 (0.52, 2.03)	0.00	144.8	−0.11 (−0.91, 0.70)	0.80
NO_2 [μg/m³]	17.7	0.59 (−0.05, 1.24)	0.07	14.1	3.03 (1.80, 4.28)	0.00	6.9	0.30 (−0.60, 1.22)	0.51

Note: *p*-Values were derived by *t* statistics. BMI, body mass index; FLC, free light chains; IQR, interquartile range; NO_2,nitrogen dioxide; $PM_{2.5}$, particulate matter with aerodynamic diameter ≤2.5 μm; PM_{10}, particulate matter with aerodynamic diameter ≤10 μm, PN_{AM}, accumulation mode particle number; SD, standard deviation.

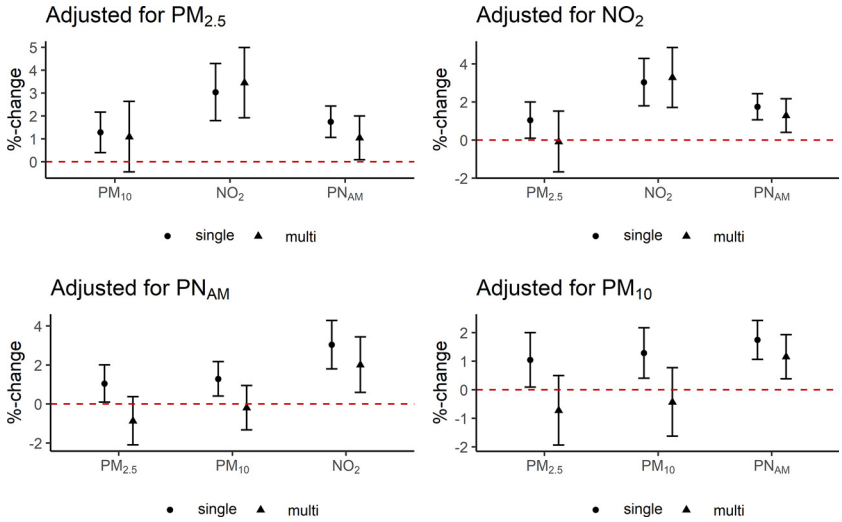

Figure 2. Associations between an interquartile range increase in 91-d mean total exposures (NO_2, $PM_{2.5}$, PM_{10}, and PN_{AM}) and free light chains in the main models including copollutants in 9,933 observations from 4,455 participants. See Table S7 for corresponding numeric data. Note: NO_2, nitrogen dioxide; $PM_{2.5}$, particulate matter with aerodynamic diameter less than or equal to 2.5 micrometers; PM_{10}, particulate matter with aerodynamic diameter less than or equal to 10 micrometers; PN_{AM} [accumulation mode particle number], accumulation mode particle number.

responses of the AIS, including antigen presentation by alveolar macrophages and dendritic cells, as well as activation of B-cells to stimulate the production of antibodies (Miyata and van Eeden 2011; Provoost et al. 2010). The proliferation of B-cells is enhanced by costimulatory molecules (for example CD40L and CD80), driven by T-cells (den Haan 2014). Other studies focus on the interplay between the IIS and the AIS in nonspecific inflammation processes, not necessarily elicited by AP. Hampson et al. (2014) points out four potential mechanisms through which FLC could interact with the IIS through neutrophils shown in *in vitro* studies. For example, apoptosis of neutrophils is hampered by FLC. Another potential pathway relating neutrophils is shown in a study combining experimental and epidemiological data and demonstrating the ability of FLC to bind to neutrophils and promote the release of inflammatory mediators (Braber et al. 2012). A cross-sectional study with different subgroups (healthy participants, chronic kidney disease patients, DM patients, and renal vasculitis and renal transplant patients, including a case series of intensive care patients) also suggests a link between hs-CRP and FLC in the inflammation process (Burmeister et al. 2014). Concurrent measurements of CRP and FLC concentrations did not correlate. However, longitudinal measurements in intensive care unit patients generally revealed a lagged response of FLC levels (starting about 5 d after the acute event), whereas hs-CRP concentrations peaked earlier (up to 5 d). By showing an effect-modifying role of hs-CRP on the association between AP and FLC, our study underlines the close relationship between the AIS and the IIS. However, clear evidence from prospective studies is still lacking.

Prior Studies Investigating Effects of AP on the Immune System

To date, most epidemiological studies have analyzed short- and long-term AP in relation to effects on (humoral) markers of the IIS such as hs-CRP interleukins and/or tumor-necrosis factor alpha (TNF-α) (Mostafavi et al. 2015; Rückerl et al. 2011; Viehmann et al. 2015; Hennig et al. 2014). Other studies examined allergic sensitization in the lung or allergy-related asthma (Grove et al. 2018). These outcomes also depend on cellular immune responses of both AIS and IIS (Kim et al. 2010) In contrast, FLC is a product of the humoral adaptive immunity, and only a few epidemiological studies have examined FLC or other markers of the humoral AIS in healthy adults (Zhao et al. 2013), children (Leonardi et al. 2000), or diabetic populations (Schneider et al. 2011). One short-term (Zhao et al. 2013) and one long-term study (Leonardi et al. 2000) generally found positive associations between $PM_{2.5}$ and antibodies, specifically IgG-type (Zhao et al. 2013; Leonardi et al. 2000), CD4+ T and CD8+ T lymphocytes (Zhao et al. 2013; Leonardi et al. 2000), B-lymphocytes (Leonardi et al. 2000), or costimulatory molecules (Schneider et al. 2011). Activation of the AIS through the NF-κB is hypothesized to up-regulate Th2-responses, stimulated by the redox reactive potential of PM components and thus initiating inflammatory and autoimmune diseases, as rheumatoid arthritis, atopic dermatitis, allergic diseases, hypersensitivity pneumonitis, chronic obstructive pulmonary disease (COPD), or multiple sclerosis (Brebner et al. 2014; Miyata and van Eeden 2011). Considering cigarette smoke exposure as a proxy or surrogate for AP exposure, an animal study found positive associations with FLC. Braber et al. (2012) observed significantly increased levels of FLC in mice exposed to cigarette smoke for 1, 4, and 20 wk.

Toxicity of Different Air Pollutants, Particle Sources, and Particle Sizes

The nature of air pollutants and the size of particles determine their toxicity and inflammatory effects. Evidence indicates that smaller particles, i.e., ultrafine particles <0.1 μm may be more toxic than larger particles (Ohlwein et al. 2019). Our results support this theory, as we observed stronger associations for (medium-termed) PN_{AM} in comparison to PM_{10} and $PM_{2.5}$. Furthermore, effect estimates were elevated for both traffic- and industry-related in comparison with total PN_{AM}. Toxicological evidence indicates that reactive oxygen species (ROS) produced by transition metals and polyaromatic hydrocarbons (PAH), which are dominant in diesel-exhaust or industry-related ultrafine particles, trigger adverse effects of immune cells, e.g., through activation of the NF-κB-factor (Dagher et al. 2007; Miyata and van Eeden 2011).

In general, we observed weaker associations with $PM_{2.5}$ in comparison with those of PM_{10}. This finding might be a consequence of the missing assimilation process for $PM_{2.5}$ in contrast

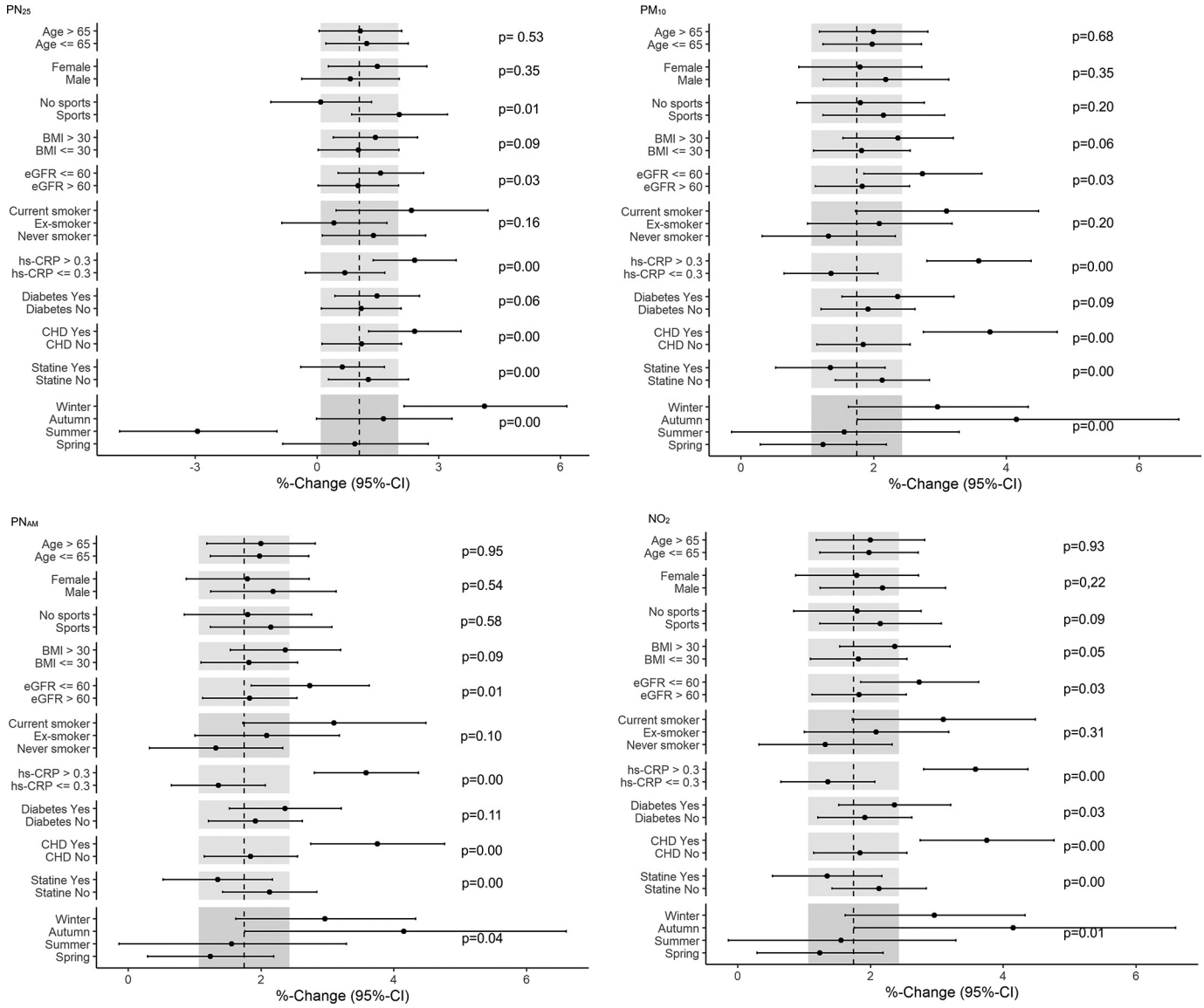

Figure 3. Effect modification by personal characteristics and season for percent change in serum free light chains per interquartile range increase in 91-d mean air pollutants. Overall effect estimates and 95% confidence intervals (CI) for particulate matter with aerodynamic diameter ≤2.5 μm [$PM_{2.5}$, 1.04 (95% CI: 0.09, 2.00)]; [PM_{10},1.28 (95% CI: 0.40, 2.17)]; accumulation mode particle number [PN_{AM}, 1.74 (95% CI: 1.06, 2.43)]; and nitrogen dioxide [NO_2, 3.03 (95% CI: 1.80, 4.28)] are shown by the vertical, dashed lines and the gray shaded areas, respectively. *p*-Values for effect modification were derived by *F* statistics. See Tables S8–S11 for corresponding numeric data.

to PM_{10}, leading to a larger exposure misclassification error for $PM_{2.5}$.

Our results show particularly increased medium-term effects for traffic-related PM in relation to total and industry-related PM. To our knowledge, only one other epidemiological study (with 110 traffic policemen in China) analyzed personally measured traffic-specific AP on markers of the AIS. This study also showing positive associations but on a short-term time scale (Zhao et al. 2013). Other studies emphasize the role of bioactive components in microbial material being dominant in larger particles such as PM_{10} (Miyata and van Eeden 2011). The content of bioactive components depends on season, temperature, and humidity. Indeed, we observed effect modification by season, showing stronger associations in winter (PM, NO_2) or autumn (PN_{AM}) vs. summer (PM, NO_2) or spring (PN_{AM}). In contrast, Zhao et al. (2013) observed higher effect estimates for IgG, IgM, and IgA and weaker effect estimates for IgE during summer. Further research is needed to explain these controversial results. In addition, there is a lack of evidence concerning the effects of NO_2 on the AIS. The few toxicological studies that investigated effects of NO_2 on inflammatory processes in the lungs refer to markers of the IIS (Mirowsky et al. 2016; Li et al. 2011; Zhu et al. 2012). A study with rodents showed that repeated exposure to NO_2-enriched exhaust from diesel with particle filter resulted in an increased number of macrophages in the alveolar tissue compared with filtered air (Karthikeyan et al. 2013). Our study suggests that both PN_{AM} and NO_2 play a crucial role for the AIS with robust associations independent of copollutants, whereas PM estimates were not independent of copollutants.

Exposure Time Windows and Other Time Aspects Determining the Association between AP and FLC

The adaptive immune system takes 4–7 d to respond to antigens and build antibodies. In healthy adults, excessively secreted FLC is cleared within 2 to 6 h (Andraud et al. 2012). In our study, we

found associations between AP and FLC being most obvious for medium-term exposures of 60-d to 120-d mean averages. Short-term effects (3-d mean) on FLC were limited to industry-related exposures. Epidemiological studies suggest that AP causes chronic effects on the IIS (Hennig et al. 2014; Viehmann et al. 2015; Rückerl et al. 2011), e.g., the thickening of the mucosal wall in the airways, fibrosis in the lung tissue, and a general modification of inflammatory processes (Feng et al. 2016), which supports the hypothesis that middle- to long-term consequences of AP seem plausible. However, the results of our long-term analyses did not show a consistent association between AP and FLC. As discussed above, the IIS and the AIS interact strongly, and we speculate that one possible explanation could be inhibitory effects between IIS and AIS. Further, associations between AP and FLC may be modified by the season. Therefore, it seems probable that specific, seasonally varying particle mixtures activate the AIS, which in turn triggers the IIS. Further research should tackle these hypotheses. An analysis stratifying for examination time (t_0, t_1, and t_2) found considerable differences with generally stronger associations at the second follow-up examinations. This difference might be partly caused by sociodemographic and health-related factors: The aging participants were mostly retired and spent more time at home and therefore had less exposure misclassification. Further, they suffered more from comorbidities. The distribution of blood draws across the seasons and differing overall exposure concentrations might also have played a role.

FLC in Vulnerable Groups

Studies in susceptible subgroups have shown associations between elevated FLC and all-cause mortality (Hutchison et al. 2014) and cardiovascular events (Bellary et al. 2014). Some studies have reported that an increase in FLC is a predictor of all-cause mortality in the general population (Dispenzieri et al. 2012; Dürig et al. 2010). Another study found, that relative risk of death, 41% of which were attributable to cardiovascular causes, increased proportionally with increased FLC concentration (Anandram et al. 2012). These results support the hypothesis, that the activation of the AIS as measured by FLC might provide an additional biological link between PM and cardiovascular diseases. Other studies found links between elevated FLC and autoimmune diseases (Bosello et al. 2018; Gottenberg et al. 2007).

Strengths and Limitations

A major strength of our study includes its population-based design in the general population, considering that most studies investigating FLC are conducted in patient populations with acute or chronic renal insufficiency or among patients referred to a clinic or a hematologist (Anandram et al. 2012). Additional strengths are that we were able to use longitudinal data with identical FLC assessment, as well as time- and spatial-dependent exposure modeling including source-specific exposure measurements for the three study visits. Nevertheless, some data that might be linked to adaptive immunity were missing (e.g., on atopy). As another limitation, the small magnitude in absolute effects preluding any clinical relevance of the FLC changes shown in our study should be mentioned. However, even small changes in the inflammation cascade may influence the fragile homeostasis of the immune system. Another limitation relates to the considerable lost to follow-up from baseline to the second follow-up. Therefore, selective attrition toward a healthier population at baseline is highly likely. Furthermore, the assessment of urban background exposure with a grid resolution of 1×1 km^2 in the densely populated Ruhr area prevented us from assessing the full exposure contrast.

Conclusion

Our study suggests that middle-term exposure to AP may modulate the immune system in a way that polyclonal FLC as a marker of the AIS is stimulated. AIS and IIS seem to interact in the immune response to AP. The specific process in this biological reaction remains mostly unknown. For the different air pollutant and time windows, the patterns of the associations are similar. Traffic- and industry-related exposures amplify the associations for the single air pollutants at different time windows.

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